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Supplementary Materials for

p63 establishes epithelial enhancers at critical craniofacial development genes

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/5/eaaw0946/DC1)

Supplemental Figures

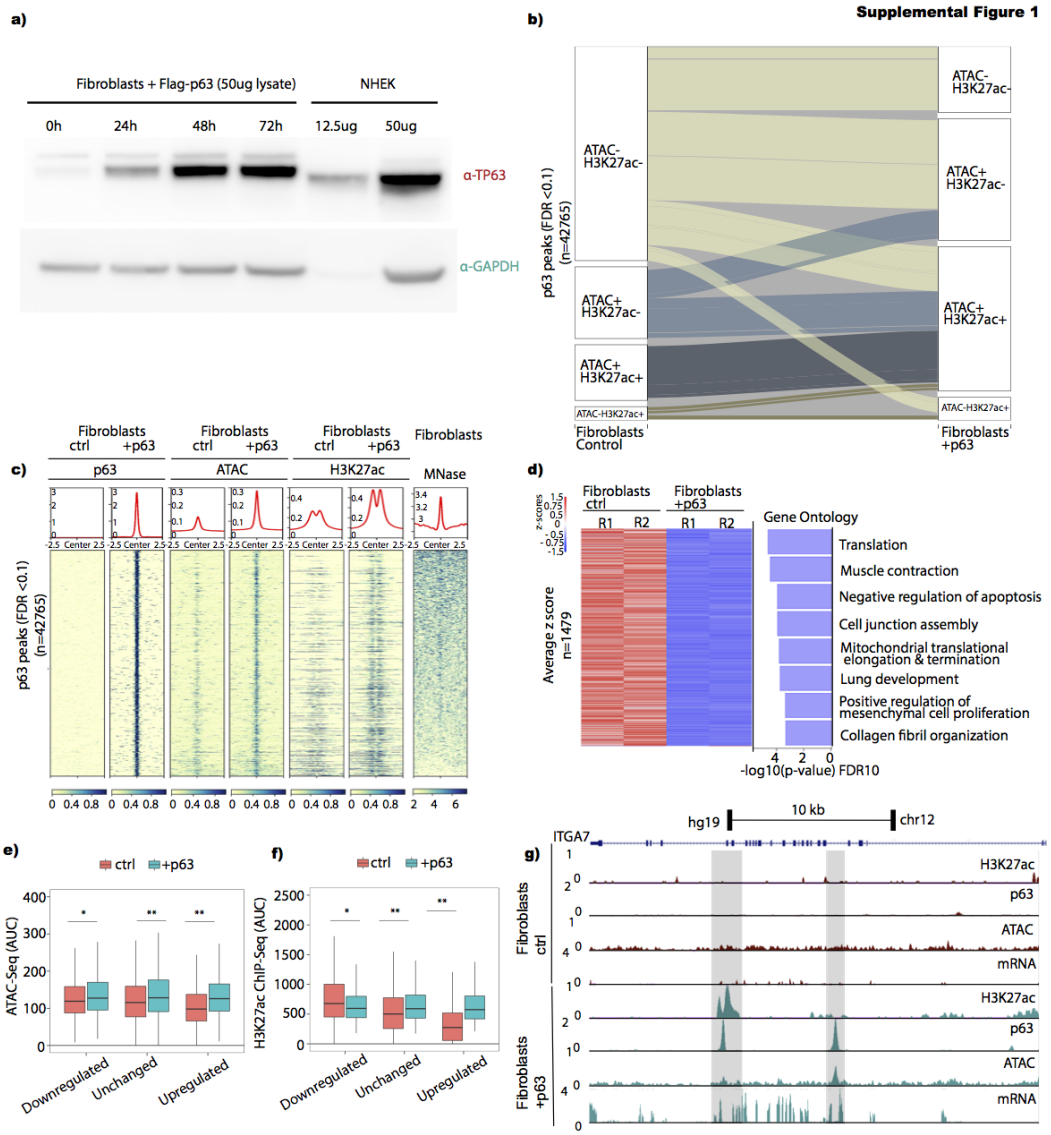

Fig. S1. Ectopic expression of p63 in fibroblasts leads to establishment of enhancers and downstream up-regulation of inflammation and epithelial genes. A) Western blot showing p63 ectopically expressed in fibroblasts reaches an expression level comparable to endogenous levels observed in undifferentiated normal human epidermal keratinocytes after 48h B) Alluvial plot depicting changes in chromatin state after ectopic expression of p63 at p63 bound peaks for chromatin transitions with >200 peaks. C) Heatmap showing high overlap between newly accessible chromatin (ATACseq) in fibroblasts + p63 and published MNaseSeq in human fibroblasts[25]. D) Heatmap showing 1479 downregulated genes in fibroblasts + p63 compared to control and GO categories enriched among downregulated genes. E)F) Boxplots showing increased chromatin accessibility and H3K27ac across downregulated, unchanged and upregulated genes in fibroblasts + p63, with the highest fold change for upregulated genes (*p-Value<10E-5, **p-Value<10E-16). G) UCSC genome browser tracks showing transcriptional activation of *ITGA7* and *de novo* H3K27ac and ATACseq signal within introns of *ITGA7* in fibroblasts with ectopic expression of p63 (grey highlighted boxes).

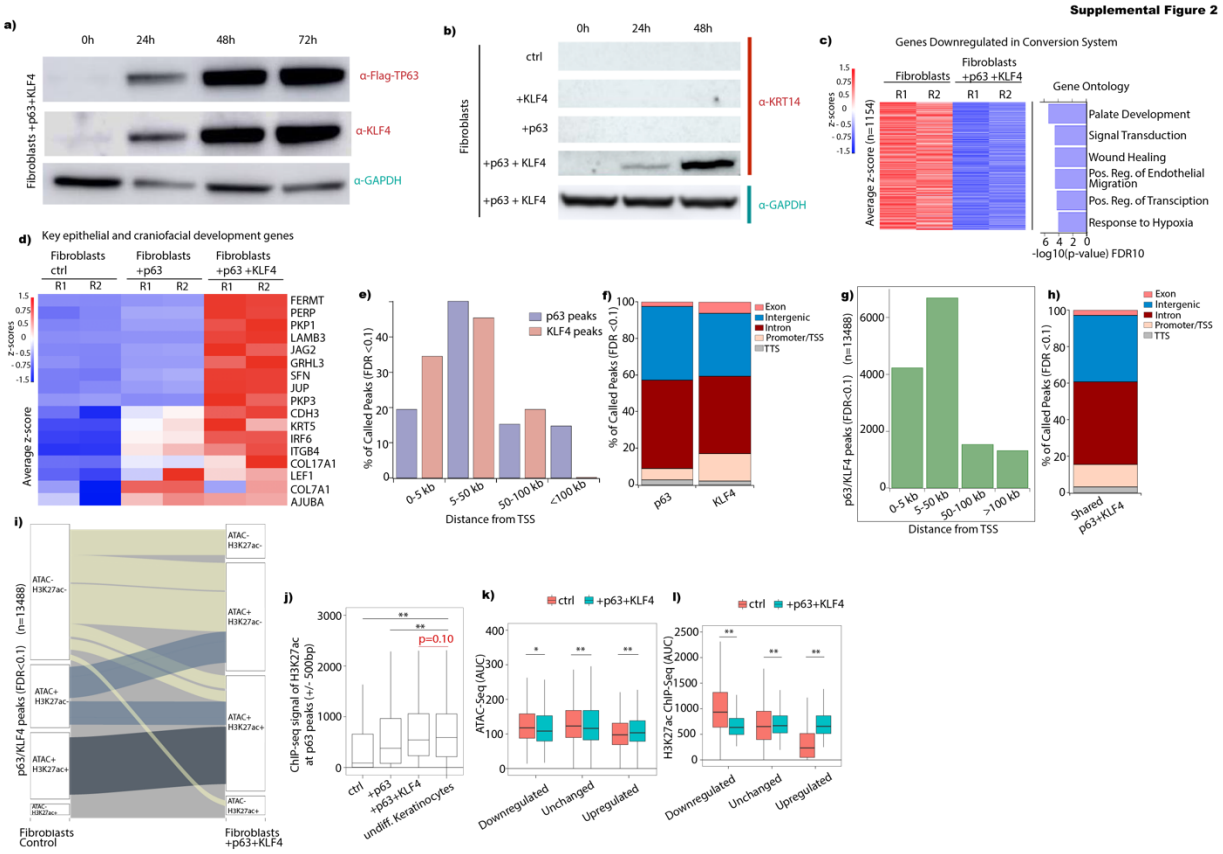

Fig. S2. Ectopic coexpression of p63 and KLF4 leads to establishment of keratinocyte-specific enhancers and up-regulation of epithelial genes. A) Western Blot showing robust co-expression of p63 and KLF4 in the inducible trans-differentiation model (fibroblasts+p63+KLF4) B) Western Blot showing KRT14 is only upregulated when both p63 and KLF4 are expressed and not in control fibroblasts, fibroblasts + KLF4 or +p63 alone. C) Heatmap showing 1154 downregulated genes in fibroblasts + p63+KLF4 compared to control and GO categories enriched among downregulated genes. D) Heatmap showing co-expression of p63 and KLF4 is required for upregulation of a subset of key epithelial and craniofacial genes. E) Distance to nearest TSS for all p63 and all KLF4 peaks in fibroblasts +p63+KLF4. F) Partitioning of p63 and KLF4 peaks into different genomic features showing intergenic and intronic regions are the most abundant for both G) Distance to closest TSS for p63/KLF4 shared peaks. H) Partitioning of 13488 p63/KLF4 shared peaks showing enrichment of intronic and intergenic regions, consistent with enhancers. I) Alluvial plot depicting changes in chromatin state after ectopic expression of p63 and KLF4 at p63/KLF4 bound peaks for chromatin transitions with >200 peaks. J) Boxplot showing H3K27ac is not statistically different (p=0.01) at shared p63 peaks between p63+KLF4 in fibroblasts and p63 in undifferentiated keratinocytes (ChIP-seq signal at p63 peaks (n=4981) +/- 500bp) (**p-Value<10E-16) K)L) Boxplots showing increased chromatin accessibility and H3K27ac only at genes upregulated in fibroblasts + p63+KLF4 (*p-Value<0.05, **p-Value<10E-16). Very high fold change in H3K27ac at p63/KLF4 peaks close to upregulated genes.

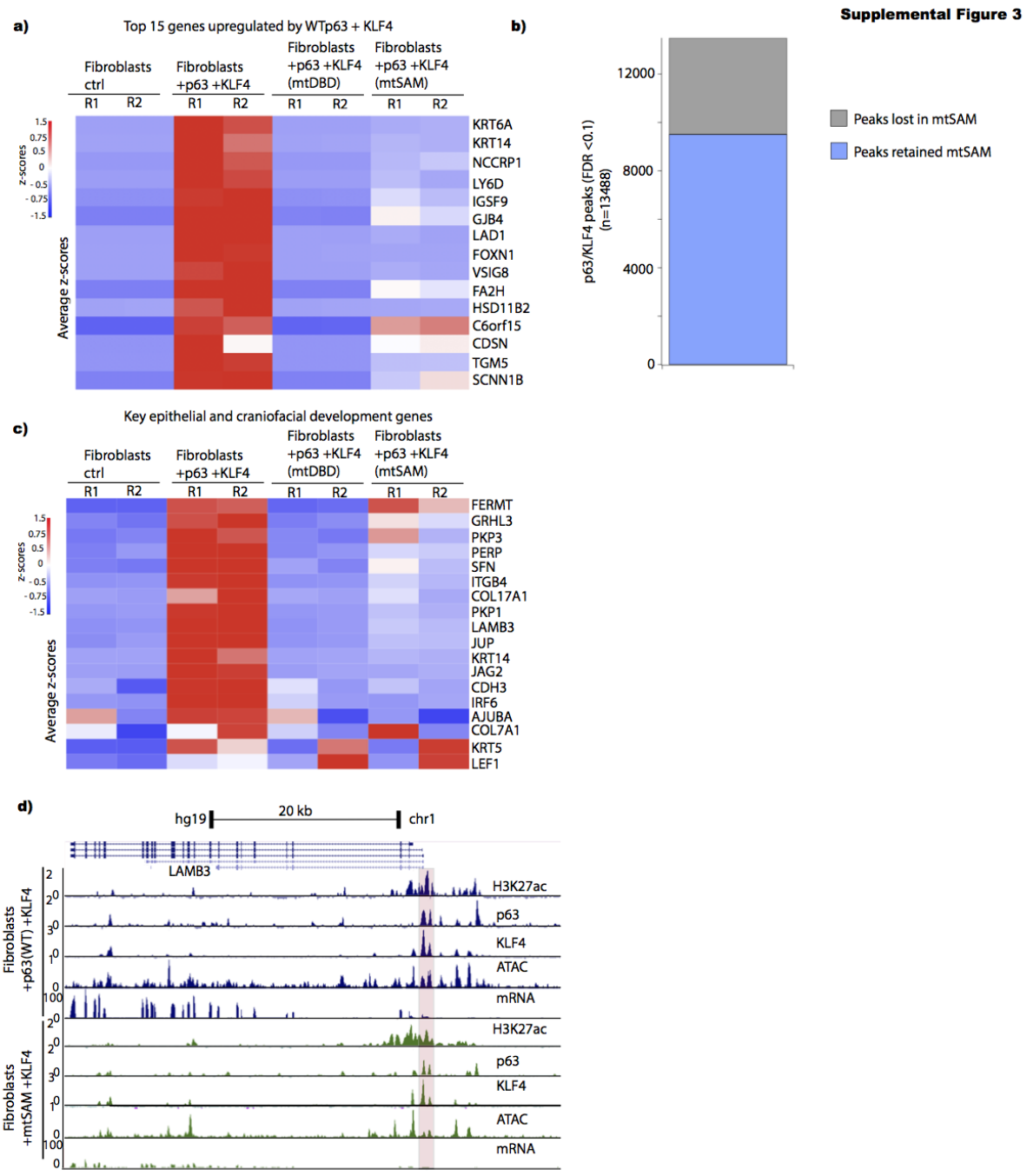

Fig. S3. Mutations in p63 disrupt enhancer establishment and lead to loss of up-regulation of key keratinocyte-specific genes. A) Heatmap showing mtDBD and mtSAM have disrupted or loss of upregulation of the top 15 genes upregulated by WTp63+KLF4. B) Category plot showing 71% (9513/13488) of p63/KLF4 sites are retained when p63 carries mutations in the SAM domain. C) Heatmap showing mtDBD and mtSAM have disrupted or loss of upregulation of a subset of epithelial and craniofacial genes. D)UCSC genome browser tracks showing defects (red box) in establishing open chromatin and inducing gene expression at *LAMB3*, a gene involved in craniofacial development.

Supplemental Figure 4

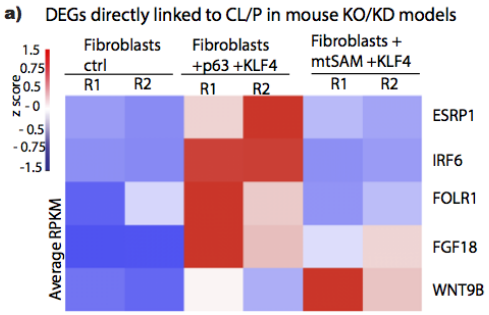

Fig. S4. Heatmap of genes regulated by p63 + KLF4 that have been strongly linked to CL/P in murine KD and KO models. A) Heatmap of genes regulated by p63+KLF4 that have been strongly linked to CL/P in murine KD and KO models.

Supplemental Figure 5

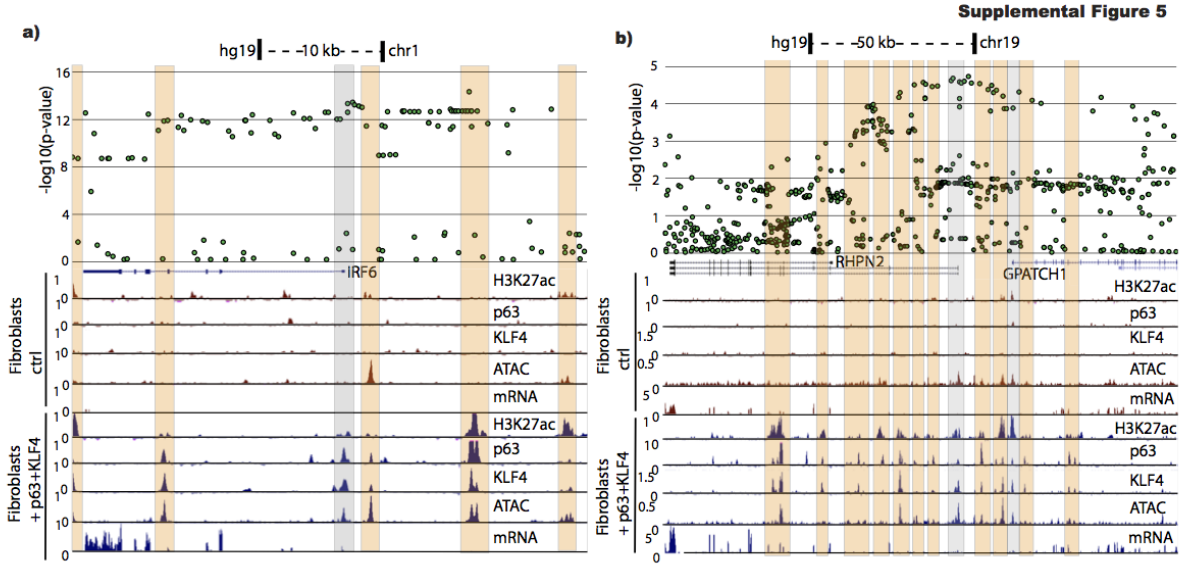

Fig. S5. p63/KLF4 peaks colocalize strongly with highly associated CL/P SNPs near *IRF6*, *RHPN2*, and *GPATCH1*. A)B) Overlay of SNPs and UCSC genome browser tracks highlighting in orange that p63/KLF4 peaks colocalize strongly with highly associated CL/P SNPs near *IRF6*, *RHPN2* and *GPATCH1*, in grey binding of both proteins at promoters.

Supplemental Table Legends

Table S1. Genes up-regulated in fibroblasts + p63 compared to control fibroblasts (fold change > 1.5; FDR < 0.05).

Table S2. Genes up-regulated in fibroblasts + p63 + KLF4 compared to control fibroblasts (fold change > 1.5; FDR < 0.05).

Table S3. Mutations in p63 disrupt up-regulation of keratinocyte-specific genes. Column 1) Genes upregulated in fibroblasts +mtDBD+KLF4 compared to control fibroblasts, column 2) Genes upregulated in fibroblasts +mtSAM+KLF4 compared to control fibroblasts, column 3) Genes no longer upregulated in fibroblasts +mtSAM+KLF4 compared to WTp63+KLF4, column 4) Newly upregulated genes in fibroblasts +mtSAM+KLF4 compared to WTp63+KLF4; fold change > 1.5 and FDR < 0.05

Table S4. Causal and candidate genes involved in CL/P identified in our transdifferentiation model. A) Representative genes involved in isolated CL/P or CPO in murine KO/KD models and transcriptional regulation in control fibroblasts, fibroblasts + WTp63+KLF4 and fibroblasts +mtSAM+KLF4 and replicates. B) List of 76 genes upregulated by p63+KLF4 identified by genetic association database. C) List of 40 known risk loci for nsCL/P and genes within the TAD domains of each loci, in orange, genes upregulated by p63+KLF4. D) List of p63/KLF4 regulated genes and respective gene based CL/P association p-values for known causal genes, genes within the 40 risk loci and genes outside of the known risk loci.